
Public Health Reports

VOLUME 64

APRIL 22, 1949

NUMBER 16

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Q Fever Studies in Southern California, III



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Q Fever Studies in Southern California

III. Effects of pasteurization on survival of *C. burnetii* in naturally infected milk

By R. J. HUEBNER, M. D., W. L. JELLISON, PH. D., M. D. BECK, and
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The first report in this series (1) described the recovery of *Coxiella burnetii* (*Rickettsia burnetii*) from the pooled raw milk of four dairies in Los Angeles County. Subsequent studies of 63 dairies in the same area have shown that the pooled raw milk of 40 dairies contained sufficient *C. burnetii* to readily infect guinea pigs on intraperitoneal or subcutaneous injection.

The second report in this series described epidemiological observations on 300 cases of Q fever (2). In that study, it was found that 68 percent of the infected persons did not use raw milk in their households. Although this observation seemed to eliminate the household use of raw milk as a mode of infection in two-thirds of the cases, it by no means eliminated the use of raw milk as a factor in the remaining third. The 32 percent of the cases which used raw milk may be compared to the less than 5 percent of the general population who are known to use raw milk in the Los Angeles area. Furthermore, it should be pointed out that case finding to date may have been influenced to some degree by selection on the basis of occupation and residence.

The resistance of *C. burnetii* to heat has been found to be much greater than that of the other rickettsias and to exceed that of most vegetative bacteria (3). This observation suggested that pasteurized milk and milk products prepared from pasteurized milk must be investigated as potential sources of Q fever infection.

The purpose of the third report in this series is to describe five controlled experiments in which the effects of two methods of pasteuriza-

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tion on the survival of *C. burnetii* in naturally infected milk were investigated. Four separate rigidly observed and controlled tests of the efficiency of each method (holding vat, and high temperature short time—HTST) were completed in the five experiments. The results of infectivity tests of market milk pasteurized in holding vats at 36 dairies are also given.

Materials and Methods

The experiments were performed in two large milk processing plants A and B. In each plant the milk used in the studies consisted of 400 to 600 gallons of pooled milk from dairies "a" and "b." Composite milk specimens from each dairy had been found consistently on several previous occasions to contain sufficient quantities of *C. burnetii* to readily infect guinea pigs.

Pasteurization Equipment

Standard holding vat pasteurizers without space heaters¹ but with vertical mixing coils were used in both plants. The Cherry-Burrell HTST pasteurizer was employed at plant A, whereas the Creamery Package HTST pasteurizer was used at plant B.

Milk Supplies

The milk supplies used in experiments I, II, and V represented the morning and evening milk of 90 to 100 cows from dairy "a". At least 13 of these cows were found shedding *C. burnetii* in milk about 2 months before these experiments were performed. The milk used in experiments III and IV represented the entire daily output of 90 cows from dairy "b," approximately 20 percent of which were positive in the complement fixation test for serum antibodies against Q fever. The milk was collected at each dairy in the customary manner² in 10-gallon cans and taken over the shortest possible route by truck (requiring 1 to 2 hours) to bottling plants A and B. At plant A the experiments were performed immediately after the evening milk of dairy "a" arrived at the plant. The morning milk supply from this dairy was kept at 40° to 43° F. until mixed with the evening milk supply when it arrived at the plant.

At plant B the experiments were performed immediately upon arrival of the morning milk shipment from dairy "b," at which time

¹ Milk ordinance and code Public Health Bulletin No. 220, 1939. Space heaters are not required by the Agricultural Code of California and are not generally used in Los Angeles.

² The milk drawn from the cows was promptly cooled to a temperature below 50° F. by pouring over standard cooling equipment. Although all cans were not tested, temperatures of those which were tested did not exceed 43° F. At dairy "a" the cans of cooled milk were placed in a cold room (temperature 40° F.) for several hours before shipment. At dairy "b" arrangements had been made for the delivery truck to arrive at the dairy as soon as all the milk had been placed in cans. This is the usual procedure at this dairy which does not have a cold room.

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it was mixed with the milk shipment of the previous evening which had been stored at 40° to 43° F. during the night.

Pasteurization Procedure

A standard procedure was designed and closely followed in each of the experiments. Prior to the pasteurizing process, the holding vats, collecting vats, pipes, and other equipment coming in contact with milk, except the HTST pasteurizers, were cleaned and subjected to live steam for 30 minutes. The HTST pasteurizer in each instance was cleaned and further treated with a chlorine solution containing several times the amount of free chlorine usually used. The solution, in turn, was fully eliminated by flowing warm water. From 400 to 600 gallons of milk were used in the experiments, depending on the amount of milk required to fill completely the holding vat pasteurizer which was also employed as a mixing vat. After 10 minutes of thorough mixing, 100 to 200 gallons of milk were routed through the HTST pasteurizer and from there, following pasteurization, to a collecting vat where the milk again was thoroughly agitated and mixed. Following completion of the HTST test the remaining milk (300 to 400 gallons) was then pasteurized directly in the holding vat where the milk had been placed originally. The operation of each pasteurizer during the experiments was regulated and observed by at least three milk inspectors representing the State, city, and county health departments.³

Pasteurization Temperature Curves

The temperatures were controlled within expected limits, except in experiment I when after completing the HTST part of the experiment insufficient milk was retained in the holding vat to control temperature variations.⁴ In no experiment did the temperature drop below the minimum legal requirements.⁵ In each of the four experiments with the HTST equipment the temperature was held for 15 seconds at 160.5° F. with a maximum variation of +0.3° F. except in experiment IV when the temperature increased for a period of less than 2 seconds to 161.5° F. Temperature curves in each holding vat pasteurization experiment were checked by thermographic records and by constant observation of thermometers immersed in the body of milk. The temperature curves of the HTST experiments were recorded exclusively on thermographs.

³ The operations in the plant were supervised by H. C. McCausland of the California State Department of Agriculture, Jack Covert and Mark Howlett of the Los Angeles City Health Department, and Dr. F. P. Wilcox of the Los Angeles County Health Department. Dr. Jacob Traum of the University of California School of Veterinary Sciences assisted in planning and observing the experiments.

⁴ This part of experiment I was not completed but was repeated successfully in experiment V.

⁵ California law requires a minimum temperature of 143° F. for 30 minutes for the holding vat and a minimum temperature of 160° F. for 15 seconds for the HTST method.

The temperature curves for the holding vat experiments are shown in table 1. The temperature was held at 143° F. with less than +0.5° F. variation for exactly 30 minutes in each experiment. However, the time lag required to bring the milk up to 143° F. was somewhat longer in each experiment than usually occurs in commercial pasteurization. This increased lag was the result of deliberate attempts to prevent excessive variation during the critical initial period when the temperature first reached 143° F.

Table 1. *Temperature curves of four milk pasteurization experiments in the holding vat*

Experiment number	Temperature of milk at start of experiment ° F.	Time lag to 143° F. (minutes)	Time lag between 140° F. to 143° F. (minutes)	Time held at 143° F. (minutes)	Temperature variation from 143° F.
II	50	50	7	30	+0.05
III	60	55	10	30	+0.02
IV	63	55	15	30	0
V	45	47	5	30	0

¹ The temperature of the milk was raised to this level in the holding vat shortly before routing it through the HTST equipment in order to reduce fluctuations of temperature in the latter. "Raw" milk specimens were taken at the temperatures shown above.

Test Specimens

Specimens to be tested for *C. burnetii* were collected as follows: Three wash-water specimens were collected in each of the experiments from the holding vat pasteurizer, the HTST pasteurizer, and the collecting vat. The specimens were collected at the end of the washing process in sterile 4-ounce screw-cap vials. The milk specimens were collected in 4-ounce screw-cap vials and in 1-quart mason jars, which were sealed and sterilized the day before the experiments were performed.

Raw milk specimens were taken from the holding vat after 10 minutes of thorough mixing of the milk shortly before the pasteurization tests were begun. Following pasteurization, milk specimens were taken from the collecting tank (HTST pasteurized specimens) and directly from the holding vat. Four-ounce and quart specimens were taken in duplicate, and the sealed jars containing hot milk were immediately placed in a pail of iced water and cooled to approximately 50° F. as required by the California agricultural code for commercial milk.

In experiments I and II, milk specimens were collected from the bottom outlet of each tank after several gallons of milk had been permitted to flow out. This technique was believed to be unwise, since raw and pasteurized milk escaped from the holding vat over the same route. This consideration apparently failed to influence the single vat experiment completed in experiment II. However, in

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experiments III, IV, and V, sterile one-pint dippers were used to obtain specimens from the central portion of milk in each of the tanks.

Processing and Testing of Specimens at the Q Fever Laboratory

In each instance when the work of collecting and cooling specimens at the bottling plant was completed, the specimens were kept iced and taken to the Q Fever Laboratory,⁴ where they were processed for inoculation in experimental animals both at that laboratory and at the National Institutes of Health, Bethesda, Maryland.

Twenty cc. amounts of milk and wash water were distributed by means of sterile pipettes from one of each of the specimen jars into at least four 30 cc. sterile screw-cap vials. Three vials representing each specimen were promptly frozen in dry ice. Five cc. of milk from each specimen were injected intraperitoneally in each of three guinea pigs. Similar amounts of wash-water specimens were also injected in two guinea pigs. At the field laboratory 90 to 100 cc. of each pasteurized milk specimen were centrifuged at 4,000 r. p. m. for 1 hour. The resulting sediments resuspended in 15 cc. of saline were divided and injected in three guinea pigs. Unopened duplicate milk specimens in quart jars were placed in the refrigerator in an upright position at 40° F. for approximately 12 hours, following which the cream was removed by pipette and placed in 20 cc. amounts in 1-ounce vials and then handled exactly as the whole milk specimens described above. Since spontaneous infections are known to occur in guinea pigs (4), uninjected animals were added to each cage as controls.

Tests at the National Institutes of Health

Specimens identical to those injected at the Q Fever Laboratory (except sediments) were shipped in dry ice by air express to the National Institutes of Health where they were kept in the frozen state until injected in laboratory animals. Guinea pigs were injected intraperitoneally with 3 cc. quantities instead of the 5 cc. amounts used at the Q Fever Laboratory. Uninjected controls were again added to each group of test guinea pigs.

Guinea Pig Inoculations

Male guinea pigs weighing 500 to 800 grams were used at both laboratories. Rectal temperatures were taken each morning on all guinea pigs. Although control guinea pigs in several experiments developed fever during the observation periods, it could not be shown that this evidence of intercurrent infections in the guinea pigs influenced the results of the experiments. The presence or absence of infection with *C. burnetii* was determined by the presence or absence

⁴ The Q Fever Laboratory, Hondo, Calif., is a temporary field laboratory supported as a cooperative undertaking by the Public Health Service, the California State Departments of Health and Agriculture, and the Los Angeles County and City Health Departments.

of specific antibodies against Q fever in the blood serum of guinea pigs 30 to 38 days after injection. The complement fixation test has been found, on extensive testing at the National Institutes of Health, to represent an accurate index of infection among guinea pigs. Because of its simplicity and accuracy, it has been used in these studies in preference to the cross immunity tests. Although intercurrent infections will frequently produce fever in guinea pigs and occasionally invalidate an immunity test, no other infectious agent has been found to stimulate the specific complement-fixing antibodies produced by *C. burnetii*. Furthermore, intercurrent infections appear to have little or no effect upon the production of such antibodies except as they may produce premature nonspecific deaths.

Additional evidence concerning infection in the guinea pigs was sought. One guinea pig in each group (except controls and guinea pigs injected with water) was sacrificed 12 to 16 days after injection. Gross autopsy findings were recorded at the Q Fever Laboratory, and second passages in guinea pigs were attempted with the autopsy tissues at the National Institutes of Health.

Mouse Inoculations

Limited numbers of mice were also injected with milk specimens at each laboratory. Although mice have been found susceptible and have been successfully employed in isolation attempts, little is known concerning the comparative value of mice as test animals for determining the presence of Q fever infection in milk. The primary purpose of the pilot-mouse experiments described in this report was to obtain information concerning the comparative sensitivities of a "mouse test" and the "guinea pig test." No attempts were made to establish strains in mice at either laboratory. Adult white and dilute brown agouti mice were injected intraperitoneally with 1 cc. of whole milk from some but not all of the experiments and placed in cages with two normal uninjected mice. Surviving mice were bled 30 days later. Because of the small quantities available the mouse serums from each group were pooled when tested by complement fixation for antibodies against Q fever.

Analysis of Milk Specimens

Chemical and bacteriological analyses of raw and pasteurized milk specimens used in each experiment are shown in table 2. The butterfat fell within expected limits and bacteria counts were not abnormal. The phosphatase test indicated that adequate pasteurization was accomplished in each experiment. However, the test was performed only upon the specimens taken from the body of the milk. No attempt was made to test foam or swabbings from the inner walls of the vat pasteurizers.

Table 2. *Chemical and bacteriological analysis¹ of the milk used in each experiment*

Experiment	Milk specimens tested	Bacterial counts	Butterfat	Acidity	Phosphatase test
I. Dairy a.	Raw milk-----	400	4.3	0.07	Positive.
	H T S T -----	300	4.3	.07	Negative.
II. Dairy a.	Raw-----	2500	4.0	Not done	Positive.
	Vat-----	300	3.7	Not done	Negative.
III. Dairy b.	H T S T -----	1200	3.9	Not done	Do.
	Raw-----	11000	3.5	0.075	Positive.
IV. Dairy b.	Vat-----	2300	3.6	.075	Negative.
	H T S T -----	2200	3.6	.08	Do.
V. Dairy a.	Raw-----	5900	3.8	.13	Positive.
	Vat-----	3200	3.8	.13	Negative.
		5100	3.8	.13	Do.
	Raw-----	} Not done		Not done	Positive.
	Vat-----	} Not done		Not done	Negative.

¹ These tests were performed in the laboratories of the Los Angeles County Health Department under the direction of Dr. R. V. Stone.

Results in Guinea Pigs

Protocols of the five experiments showing results obtained in guinea pigs are given in tables 3 to 7. Since in all the experiments injections of milk, cream, and sediment specimens gave results which were similar in all respects, the tables do not show this breakdown. Some of the guinea pigs which were injected did not survive to be tested in the complement fixation test. A few died before bleeding, and others were autopsied following which tissues were either passed into other guinea pigs or frozen for possible future use.

Table 3. *Pasteurization experiment I. Results in guinea pigs injected at 2 laboratories*

Laboratory and date specimens injected	Specimens injected	Number guinea pigs injected	Number guinea pigs developing fever	Number positive ¹ in complement fixation test	Number negative ² in complement fixation test	Number not tested in complement fixation test	Comments on (col. 7) guinea pigs not tested by complement fixation
Q. Fever Laboratory, Nov. 26, 1947.	Raw milk-----	9	8	4	1	4	2 died on 19th and 20th days. 1 passed—2d passage positive for Q fever. 1 autopsied 17th day. Spleen enlarged. 3 autopsied on 18th day. Spleen normal.
	H T S T pasteurized milk.	9	1	0	6	3	
	Wash water-----	6	1	1	5	0	
	Controls-----	6	0	0	6	0	
National Institutes of Health, Dec. 1, 1947.	Raw milk-----	8	7	3	0	5	1 died on 4th day. 4 autopsied in moribund state. 2 showed evidence of intercurrent bacterial infection. 2 autopsied—2d passage negative. 2 autopsied and not passed. 1 died on 29th day. 2 died on 9th and 25th days, respectively.
	H T S T pasteurized milk.	8	7	0	4	4	
	Wash water-----	12	3	0	11	1	
	Controls-----	14	5	0	12	2	

¹ Guinea pigs with morning rectal temperatures greater than 39.5° C. on one or more days during the observation period except the day after injection.

² Guinea pigs were bled 30 to 38 days after injection.

Table 4. Pasteurization experiment II. Results in guinea pigs injected at 2 laboratories

Laboratory and date specimens injected	Specimens injected	Number guinea pigs injected	Number guinea pigs developing fever	Number positive ¹ in complement fixation test	Number negative ² in complement fixation test	Number not tested in complement fixation test	Comments on (col. 7) guinea pigs not tested by complement fixation
Q Fever Laboratory, Nov. 27, 1947.	Raw milk.....	6	6	3	1	2	1 autopsied 19 days after injection. Spleen 3× normal. 1 died 9 days after injection. Spleen normal.
	Vat pasteurized milk.....	9	2	0	7	2	1 died 19 days after injection. Spleen normal—pneumonia. 1 autopsied 19th day. Spleen normal.
	HTST pasteurized milk.....	8	4	0	6	2	2 autopsied 19 days after injection. Spleens normal.
	Wash water.....	6	2	0	6	0	
	Controls.....	7	0	0	7	0	
National Institutes of Health, Dec. 17, 1947.	Raw milk.....	6	6	5	1	0	1 autopsied on 16th day. Passage negative. 1 died on 22d day.
	Vat pasteurized milk.....	6	0	0	4	2	
	HTST pasteurized milk.....	3	2	0	1	2	1 died 21st day. Spleen covered with nodules. 1 autopsied on 16th day. Passage negative.
	Wash water.....	6	0	0	6	0	
	Controls.....	6	0	0	5	1	1 died on 23d day.

¹ Guinea pigs with morning rectal temperatures greater than 39.5° C. on one or more days during the observation period except the day after injection.

² Guinea pigs were bled 30 to 38 days after injection.

Table 5. Pasteurization experiment III. Results in guinea pigs injected at 2 laboratories

Laboratory and date specimens injected	Specimens injected	Number guinea pigs injected	Number guinea pigs developing fever	Number positive ¹ in complement fixation test	Number negative ² in complement fixation test	Number not tested in complement fixation test	Comments on (col. 7) guinea pigs not tested by complement fixation
Q Fever Laboratory, Dec. 3, 1947.	Raw milk.....	6	5	5	1	0	
	Vat pasteurized milk.....	9	1	0	6	3	3 autopsied on 18th day. Spleens normal.
	HTST pasteurized milk.....	9	4	0	6	3	2 autopsied on 18th day. Spleens normal. 1 autopsied on 18th day. Spleen 2× normal.
	Wash water.....	6	2	0	6	0	
	Controls.....	8	3	0	8	0	
National Institutes of Health, Dec. 1, 1947.	Raw milk.....	6	6	4	0	2	2 passed—2d passage positive for Q fever.
	Vat pasteurized milk.....	6	0	0	3	3	1 autopsied on 18th day.
	HTST pasteurized milk.....	6	1	0	3	3	1 passed on 17th day—2d passage negative. 1 died on 17th day.
	Wash water.....	6	3	0	6	0	1 died on 18th day. 2 autopsied on 18th day. 1 passed—2d passage negative.
	Controls.....	6	0	0	6	0	

¹ Guinea pigs with morning rectal temperatures greater than 39.5° C. on one or more days during the observation period except the day after injection.

² Guinea pigs were bled 30 to 38 days after injection.

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Table 6. Pasteurization experiment IV. Results in guinea pigs injected at 2 laboratories

Laboratory and date specimens injected	Specimens injected	Number guinea pigs injected	Number guinea pigs developing fever ¹	Number positive ² in complement fixation test	Number negative ³ in complement fixation test	Number not tested in complement fixation test	Comments on (col. 7) guinea pigs not tested by complement fixation
Q. Fever Laboratory, Dec. 5, 1947.	Raw milk-----	9	7	6	0	3	1 died on 13th day. Spleen 3X normal. 1 autopsied on 18th day. Spleen 4X normal. 1 autopsied on 18th day. Spleen normal.
	Vat pasteurized milk.	8	0	0	5	3	3 autopsied on 18th day. Spleens normal.
	H T S T pasteurized milk.	9	0	0	6	3	2 autopsied on 18th day. Spleens normal. 1 died in 3 days—peritonitis.
	Wash water---	6	1	0	5	1	1 died in 3 days—peritonitis.
	Controls-----	10	1	0	9	1	1 autopsied on 18th day. Spleen normal.
National Institutes of Health, Dec. 12, 1947.	Raw milk-----	6	5	4	0	2	2 passed in 12 days. 2d passage positive.
	Vat pasteurized milk.	6	2	0	3	3	1 autopsied on 18th day. 1 died on 18th day. 1 passed on 17th day—2d passage negative.
	H T S T pasteurized milk.	6	1	0	4	2	1 autopsied on 10th day 1 passed on 17th day—2d passage negative.
	Wash water---	6	0	0	6	0	
	Controls-----	6	1	0	6	0	

¹ Guinea pigs with morning rectal temperatures greater than 39.5° C. on 1 or more days during the observation period except the day after injection.

² Guinea pigs were bled 30 to 38 days after injection.

Table 7. Pasteurization experiment V. Results in guinea pigs injected at 2 laboratories

Laboratory and date specimens injected	Specimens injected	Number guinea pigs injected	Number guinea pigs developing fever	Number positive ² in complement fixation test	Number negative ³ in complement fixation test	Number not tested in complement fixation test	Comments on (col. 7) guinea pigs not tested by complement fixation
Q. Fever Laboratory Dec. 9, 1947.	Raw milk-----	{ 6 + 6	{ Not taken 6	4 5	0 0	2 1	{ 2 died on 15th day. Spleen 4X normal. 1 died on 6th day—pneumonia.
	Vat pasteurized milk.	{ 9 + 6	{ 4 2	1 0	4 6	4 0	{ 3 autopsied on 16th day. Spleens normal. 1 died on 26th day. Spleen normal.
	Wash water---	2	0	0	2	0	
	Controls-----	{ 4 + 4	{ 1 0	0 0	5 4	0 0	
National Institutes of Health Dec. 12, 1947.	Raw milk-----	6	5	5	0	1	1 died on 19th day. No observation recorded.
	Vat pasteurized milk.	9	3	2	7	0	
	Wash water---	3	0	0	3	0	
	Controls-----	5	0	0	5	0	

¹ Guinea pigs with morning rectal temperatures greater than 39.5° C. on one or more days during the observation period except the day after injection.

² Guinea pigs were bled 30 to 38 days after injection.

³ Specimens injected into guinea pigs on Jan. 24, 1948.

Summarized in table 8 are the results of complement fixation tests on the serums of guinea pigs which survived injection with specimens from five experiments. Of 52 guinea pigs injected with raw milk, 48, or 92.3 percent, were positive in the complement fixation test at a serum dilution of 1 to 64 or greater. All 36 guinea pigs injected with HTST pasteurized milk were negative at a dilution of 1 to 8. Of 48 guinea pigs injected with vat pasteurized milk 3, or 6.2 percent, were positive. All 3 of the "positive" guinea pigs

Table 8. *Summary of complement fixation tests on guinea pigs used in 5 pasteurization experiments at 2 laboratories*

Material injected	Number guinea pigs	Number positive ¹	Percent positive ¹
Raw milk.....	52	¹ 48	92.3
HTST pasteurized milk.....	36	² 0	0
Holding vat pasteurized milk.....	48	¹ 3	6.2
Wash water.....	57	¹ 1	1.7
None (controls).....	73	0	0

¹ All positive serums revealed titers greater than 1:64.

² 0 = Negative at a dilution of 1:8.

occurred among 20 which were tested following injection with vat pasteurized milk in experiment V (table 7). One of these was tested at the Q Fever Laboratory and two were tested at the National Institutes of Health. Each of the three "positive" guinea pigs had febrile reactions with onsets 8, 10, and 14 days, respectively, after injection. Three of the seventeen "negative" guinea pigs injected with the same specimen also developed fever but in one the fever began on the day after injection and was limited to a single day in the other two. Three other guinea pigs also showed fever but did not survive to be tested for serum antibodies against Q fever.

No satisfactory explanation was found to account for the occurrence of antibodies in one guinea pig injected with wash water from the HTST pasteurizer (experiment I) and tested at the Q Fever Laboratory. Fifty-six other guinea pigs injected with wash water and 73 uninjected control guinea pigs were tested during the five experiments at both laboratories, and all were negative.

Five attempts to produce Q fever in second passage guinea pigs with tissues of guinea pigs injected with raw milk from experiments I, III and IV were in each instance successful. Eight similar attempts with pasteurized milk from experiments I, II, III, and IV were unsuccessful.

Retrospective attempts to establish strains from the pasteurized milk of experiment V several months after the original results were obtained were not successful. However, this was not unexpected since the specimens had been frozen and thawed several times and maintained at a freezing temperature greater than -10° C.

The febrile reactions observed in guinea pigs during the five experiments are shown in table 9. In a previous study (1) it was noted that 26 (90 percent) of 29 milk specimens infected with *C. burnetii* produced fever in guinea pigs. Similarly in this study, 61 (89.7 percent) of 68 guinea pigs injected with raw milk developed febrile reactions (table 9). After pasteurization, milk injections produced fewer febrile reactions but the number of these reactions exceeded expected rates. However, febrile reactions during these studies

Table 9. *Febrile¹ reactions in test and control guinea pigs during 30 to 38 days' observation*

Material injected	Number guinea pigs	Number showing fever	Percent showing fever
Raw milk.....	68	61	89.7
HTST pasteurized milk.....	58	20	34.5
Holding vat pasteurized milk.....	68	14	20.3
Wash water.....	59	12	20.3
None (controls).....	77	11	14.3

¹ Elevation of morning temperature above 39.5° C. for one or more days except the day after injection.

were influenced to some extent by intercurrent infections since 14.3 percent of uninjected controls developed fever. That 20 of 58 (34.5 percent) guinea pigs injected with milk pasteurized in the HTST equipment developed febrile reactions was not accorded much significance since this high percentage was largely due to the fact that the guinea pigs used in experiment I at the National Institutes of Health showed a very high rate of intercurrent infections.

Results in Mice

The results of milk injection of mice in general were similar to those obtained in guinea pigs and further demonstrated the efficacy of

Table 10. *Complement fixation results on serums of mice bled 30 days after injection with raw and pasteurized milk specimens*

Laboratory	Experiment number	Serums of mice injected with raw milk		Serums of mice injected with vat pasteur- ized milk		Serums of mice injected with HTST pasteur- ized milk		Serums of un- injected control mice	
		Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
Q fever laboratory ¹	II	2	0	2	0	2	0	5	0
	III	2	2	2	0	2	0	6	0
	IV	1	1	2	0	2	0	6	0
National Institutes of Health ²	II	2	2	1	0	1	0	1	0
	III	2	2	1	0	1	0	1	0
	IV	1	1	1	0	1	0	-----	-----
Total.....		10	8	8	0	8	0	19	0

¹ Each serum specimen represents 1 or 2 mice.

² Each serum specimen represents pooled serums of 2 or 3 mice.

pasteurization in eliminating *C. burnetii* from milk. Of ten groups of mice injected at both laboratories with raw milk from three experiments, eight were positive when pooled serums representing each group taken 30 days after injection were examined in the complement fixation test for antibodies against Q fever (table 10). Sixteen groups of mice injected with vat and HTST pasteurized milk specimens were in every instance negative when similarly tested. Nineteen groups of uninjected controls were also negative. No difference was observed between the reactions of the two strains of mice (dba and Swiss). Unfortunately, specimens from experiment V were not tested in mice.

Tests of Market Milk Specimens

As a result of findings in experiment V, further tests of vat pasteurized milk appeared indicated. Thirty-two bottles of vat pasteurized whole milk and four bottles of vat pasteurized cream were obtained from Los Angeles markets and the standard amounts injected in guinea pigs at the Q Fever Laboratory. Three specimens of whole milk and one cream specimen produced serological evidence of Q fever in at least one of two guinea pigs.

Although the phosphatase test indicated that one of the "positive" milk specimens was not free of the phosphatase enzyme and therefore probably was not fully pasteurized according to regulations, the other "positive" specimens were negative in the phosphatase test and apparently were properly pasteurized.

Discussion

Recent and contemporary reports (5) have shown that Q fever is a disease entity of some consequence to man in this and other countries. Although most reported cases cannot be traced to personal or household use of infected milk, the demonstration of *C. burnetii* infection in the mammary gland of cows (1), goats (6), and sheep (6) may indicate that milk represents a reservoir of Q fever infection. In previous but as yet unpublished work (3), it was found that *C. burnetii* in laboratory suspensions (saline and skim milk) showed considerable resistance to heat; surviving a temperature of at least 60° C. for 30 minutes in sealed vials. Since this is only slightly below California minimum requirements⁷ for the holding vat method of pasteurization and since this type of commercial pasteurization cannot provide assurance that each particle of milk is raised to the recorded temperature, additional work needs to be done in order to determine methods of pasteurization which are completely effective in eliminating *C. burnetii* from milk.

⁷ Agricultural Code of California, September 19, 1947.

Summary

Four rigidly observed tests of the holding vat and the high temperature fast time (HTST) techniques of pasteurization, as employed in the Los Angeles area were completed in five separate experiments. The presence or absence of infection was determined chiefly by serum reactions in the complement fixation test for Q fever shown by guinea pigs and mice 30 days or more after being injected with the milk specimens.

So far as could be determined by the methods available, milk supplies pasteurized in the HTST equipment were rendered free of infection, although the same milk in the raw state was shown in each instance to be highly infectious.

In three of the four vat pasteurization experiments using the holding vat without space heater, *C. burnetii* apparently was eliminated from the infected milk supplies. However, the pasteurized milk from one of the four tests of the holding vat method produced antibodies against Q fever in 3 of 20 guinea pigs tested. Similarly 3 of 32 specimens taken from bottles of vat pasteurized market milk and 1 of 4 specimens of vat pasteurized market cream produced antibodies against Q fever when injected in guinea pigs.

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- (2) Beck, M. D., Bell, J. A., Shaw, E. W., and Huebner, R. J.: Q fever studies in Southern California. II. An epidemiological study of 300 cases. Pub. Health Rep. **64**: 41-56 (1949).
- (3) Huebner, R. J.: Unpublished data.
- (4) Huebner, R. J.: Report of an outbreak of Q fever at the National Institute of Health. II. Epidemiological features. Am. J. Pub. Health **37**: 431-440 (1947).
- (5) Huebner, R. J., Jellison, W. L., and Beck, M. D.: Q fever, a review of current knowledge. (To be published.)
- (6) Caminopetros, J.: Q fever (Balkan grippé) Abstracts of Fourth International Congress on Tropical Medicine and Malaria, Washington, D. C., May 10-18, 1948, pp. 33-34.

—Announcement—

Formation of the American Board of Preventive Medicine and Public Health, Inc.

The American Board of Preventive Medicine and Public Health, Inc., has been formed to further preventive medicine and public health and to issue to physicians certificates of special knowledge in this field.

Organization of the Board was accomplished by a nine-member committee appointed from five associations. Membership included three members appointed from the Section on Preventive and Industrial Medicine and Public Health of the American Medical Association; three appointed by the Executive Board of the American Public Health Association, and one each appointed by the Canadian Public Health Association, the Southern Medical Association, the Association of Schools of Public Health.

Three additional members of the Board, representing practitioners of the specialty preventive medicine and public health, were elected following incorporation.

The principal purposes of the board as defined in the articles of incorporation are:

1. To encourage the study, improve the practice, elevate the standards and advance the cause of preventive medicine and public health.
2. To grant and issue to physicians duly licensed by law to practice medicine certificates of special knowledge in preventive medicine and public health.

General Requirements for Certification

1. Moral and ethical standing in the profession satisfactory to the Board.
2. Graduation from a medical school in the United States or Canada approved by the Council on Medical Education and Hospitals of the American Medical Association, or from a foreign medical school satisfactory to the Board.
3. An internship of at least one year in a hospital approved by the Council on Medical Education and hospitals of the American Medical Association or in a foreign hospital satisfactory to the Board.
4. Licensure to practice medicine in the United States or the Dominion of Canada.
5. Special training in preventive medicine or public health which shall include:
 - (a) A period after internship of not less than 6 years of special training, teaching or practice in preventive medicine and public health which must include (b) and (c).

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- (b) Successful completion of at least one academic year of graduate study leading to the degree of Master of Public Health or an equivalent degree or diploma or an equivalent satisfactory to the Board.
- (c) Field training or residency of at least 2 years of field experience in general public health practice which includes planned instruction, observation and active participation in a comprehensive, organized, public health program, one year of which may be an approved clinical residency in a field directly related to public health.
6. Limitation of practice to teaching or practice of preventive medicine or public health as a specialty.

The Founder's Group

The bylaws authorize the Board for a limited period of time to excuse from examination practitioners of preventive medicine or public health who have attained unquestioned eminence in the field. This has been so defined as to include professors and associate professors of preventive medicine or public health in medical schools approved by the Council on Medical Education and Hospitals of the American Medical Association or in schools of public health accredited by the American Public Health Association, or individuals who have been or are president of one of the sponsoring societies designated in the certificate of incorporation, or have had at least 10 years of distinguished service in the field of public health and are considered eligible by the Board. Applications for consideration as members of the Founder's Group must be received by July 1, 1950.

Examinations

Examinations will be held from time to time and in various places depending upon need as indicated by applications received. It is expected that examinations will be held primarily in connection with the meetings of the American Public Health Association. The examination will consist of two parts:

Part one will consist of a comprehensive written examination designed to test the knowledge of the applicant in the general field of preventive medicine and public health. These examinations will be announced at least 60 days prior to the holding of the examinations. The written examination will be confined to one day. Part two will consist of the oral or practical examination which will be held on the day following the written examination. This examination will be held before two to four examiners, members of the Board or associate examiners. An endeavor will be made to adapt the details of the oral examination to each candidate's experience and practice.

The examiners will report orally upon each candidate to the assembled Board, after which the results of the examination will be considered jointly by the entire Board and the examiners. Final action of the Board will be based upon the candidate's ethical and professional record, training and attainments as well as on the results of his formal examination.

Applications

Each application for examination for certification shall be in writing signed by the applicant and shall be filed with the secretary* not less than 90 days prior to the date of examination. Application must be on prescribed application forms. The application must be accompanied by an application fee and two recent, clear, unmounted, autographed photographs of the applicant and by at least two letters from physicians duly licensed by law to practice medicine, stating in substance that in the opinion of the writer of such letter the applicant is eligible under the provisions set up by the Board.

Fees

The Board is incorporated as a nonprofit corporation and no member of the Board may receive any compensation. Minimum fees are specified, consistent with the cost of examination and certification. The total cost to the applicant is \$50.

Application fee ----- \$15

No application can be considered for classification and action by the eligibility committee unless accompanied by the application fee. The application fee is not refundable.

Certification fee ----- \$35

This fee is payable when the candidate is notified of acceptance for examination or as a member of the Founder's Group. It is not returnable after the candidate has been officially accepted and notified by the eligibility committee to report for examination. All applications must be on the standard form and accompanied by the necessary documentation. No member of the Board is authorized to give informal opinions as to the eligibility of the candidates. The determination of eligibility will be made only by the eligibility committee after receiving full application information. All candidates must comply with Board regulations in effect for the year in which the examination is taken, regardless of when the original application was filed. Applicants declared ineligible for admission to examination may reopen their applications within 2 years of the filing date without payment of an additional application fee. Applicants declared

* Ernest L. Stebbins, M. D., 615 North Wolfe Street, Baltimore 5, Md.

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eligible but who fail to exercise the examination privilege within 3 years of the date of the filing of the application are required to file a new application and to pay a new application fee.

Certification

Upon satisfactory completion of the examinations or in the case of the Founder's Group, upon the affirmative vote of the Board, a certificate will be issued to the effect that the person named has been found to be possessed of special knowledge in preventive medicine and public health. This certificate will be signed by the officers of the Board and shall have its seal affixed. Each certificate shall remain the property of the corporation, but each person to whom a certificate is issued shall be entitled to its possession until revoked. Any certificate issued by the Board may be revoked if evidence, satisfactory to the Board, is presented that the applicant was not eligible at the time of application, or made any misstatement or misrepresentation of facts or that his license to practice medicine has been suspended or revoked.

The Board was created in accordance with action of the Advisory Board for Medical Specialties and approved by the Council on Medical Education and Hospitals of the American Medical Association.

DEATHS DURING WEEK ENDED MAR. 26, 1949

[From the Weekly Mortality Index, issued by the National Office of Vital Statistics]

	Week ended Mar. 26, 1949	Correspond- ing week, 1948
Data for 94 large cities of the United States:		
Total deaths	10,146	9,703
Median for 3 prior years	9,703	
Total deaths, first 12 weeks of year	118,822	123,111
Deaths under 1 year of age	641	688
Median for 3 prior years	681	
Deaths under 1 year of age, first 12 weeks of year	8,042	8,357
Data from industrial insurance companies:		
Policies in force	70,534,114	71,146,501
Number of death claims	12,813	13,380
Death claims per 1,000 policies in force, annual rate	9.5	9.8
Death claims per 1,000 policies, first 12 weeks of year, annual rate	9.5	10.6

INCIDENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

REPORTS FROM STATES FOR WEEK ENDED APRIL 2, 1949

A net decline of 1,442 cases in the incidence of measles was reported—from a total of 33,347 last week to 31,905 currently. The 5-year (1944-48) median is 23,784, reported for the corresponding week last year. Decreases in 5 geographic areas, ranging from 453 in the South Atlantic to 754 in the Middle Atlantic, aggregating 3,040, were partly offset by increases reported in the New England area (from 3,209 last week to 3,670), the East North Central (3,135 to 3,539), the Mountain (1,130 to 1,340), and the Pacific (2,759 to 3,282). Of 24 States reporting more than 346 cases, 13 reported increases, the largest of which occurred in California (1,833 to 2,230), New Jersey (1,366 to 1,663), Wisconsin (1,700 to 1,951), Washington (411 to 651), and Massachusetts (1,158 to 1,391). The total to date is 283,056, 5-year median 199,206. Only 4 times in the past 13 years has the reported peak of weekly incidence occurred before the first week of April.

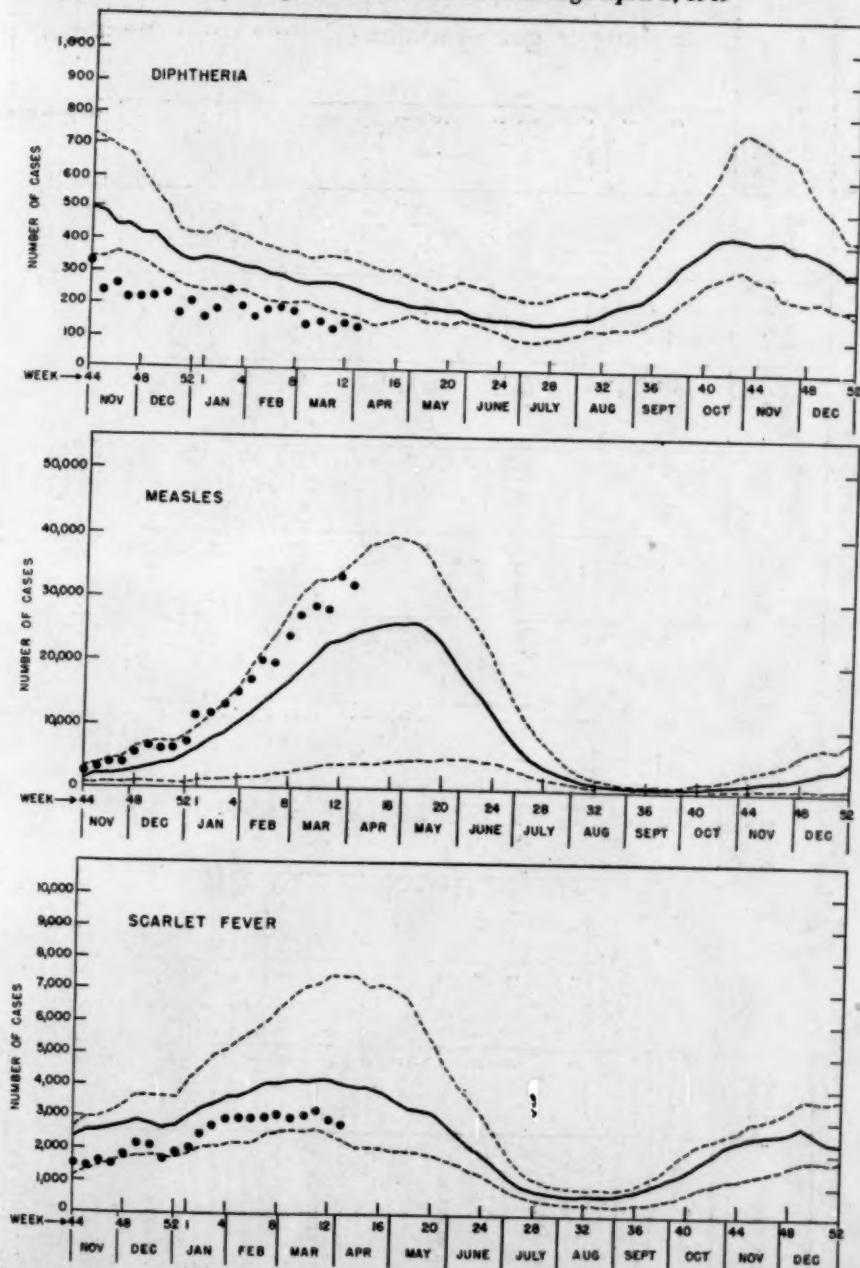
A total of 3,068 cases of influenza was reported, last week 3,616, 5-year median 2,770. Only Virginia (265), South Carolina (418), and Texas (1,495), reported more than 163 cases.

Of 43 cases of poliomyelitis, only 4 States reported more than 2 cases each—California 11, Texas 6, and Montana and Utah 5 cases each. The total to date is 1,016, 5-year median 453, corresponding period in 1947 (highest of the past 5 years) 667.

During the week 3 cases of anthrax were reported, 2 in New York and 1 in Massachusetts, and 2 cases of smallpox, 1 each in Mississippi and Texas. Since the first of the year 3 cases of smallpox formerly reported (1 each in Louisiana, South Dakota, and Wisconsin) have been canceled, leaving the corrected total to date 25, as compared with 33 for the same period last year and a 5-year median of 124.

Deaths recorded during the week in 94 large cities in the United States totaled 9,819, as compared with 10,146 last week, 9,742 and 10,248, respectively, for the corresponding weeks of 1948 and 1947, and a 3-year (1946-48) median of 9,742. The total to date is 128,641, as compared with 132,853 for the same period last year. Infant deaths totaled 654, last week 641, 3-year median 677. Cumulative figure, 8,696, same period last year 9,039.

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Communicable Disease Charts*All reporting States, November 1948 through April 2, 1949*

The upper and lower broken lines represent the highest and lowest figures recorded for the corresponding weeks in the 7 preceding years. The solid line is the median figure for the 7 preceding years. All three lines have been smoothed by a 3-week moving average. The dots represent numbers of cases reported for the weeks of 1948.

Telegraphic case reports from State health officers for week ended Apr. 2, 1949

[Lenders Indicate that no cases were reported]

Division and State	Diphtheria	Influenza	Measles	Meningitis, meningo-coccal	Pneumonia	Poliomyelitis	Rocky Mt. spotted fever	Scarlet fever	Small-pox	Tuberculosis	Typhoid and para-typhoid fever*	Whooping-cough	Rabies in animals
NEW ENGLAND													
Maine.....		30	630	16	22					1	13		
New Hampshire.....		11	32	3	12								
Vermont.....			322		3								
Massachusetts.....	10	1,391	1,316	10	238					1	14		
Rhode Island.....					8						2		
Connecticut.....	1	1	5	979	1	67		32					
MIDDLE ATLANTIC													
New York.....	3	1	24	2,825	7	399	2			5	162	2	
New Jersey.....	4	3	(*)	1,663	1	75				2	41	2	
Pennsylvania.....	2	4		2,237	8			204					
EAST NORTH CENTRAL													
Ohio.....	2	2	1	567	4	52	2			2	69	25	
Indiana.....	2	22	1	283	8	8				1	17	18	
Illinois.....			127	3	364	1		140		1	32	1	
Michigan.....	1	7	611	4	99			430			22		
Wisconsin.....		2	32	1,951	1	13		63			15		
WEST NORTH CENTRAL													
Minnesota.....	6			125	2	24	1		73				
Iowa.....	1			83					13				
Missouri.....	1	5		296		2			11		2		
North Dakota.....		11	38						4				
South Dakota.....			10	1					11		9		
Nebraska.....		18	88			2	1		5				
Kansas.....	4		1,363			5			30				
SOUTH ATLANTIC													
Delaware.....		1	10							25		2	
Maryland.....	1	4	893					41				9	
District of Columbia.....			161					23					
Virginia.....	3		265	1,363	3	81						22	4
West Virginia.....			55	68	979	2		4				2	
North Carolina.....	5										23	10	
South Carolina.....	6	1	418	622	1						13	2	
Florida.....	3	22									10	1	
	6										6	1	
											6	4	

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	EAST SOUTH CENTRAL	WEST SOUTH CENTRAL	MOUNTAIN	PACIFIC
Kentucky	3	1	1	1
Tennessee	5	250	7	37
Alabama	10	94	5	28
Mississippi *	1	163	2	17
		634	1	1
		98		
Arkansas	4	118	1,051	(1)
Louisiana	4	5	40	8
Oklahoma		28	421	4
Texas	26	1,405	3,401	20
		1	10	1
		470	6	42
Montana		15	135	18
Idaho	2	17	159	24
Wyoming		159	10	2
Colorado	10	45	66	12
New Mexico		1	302	6
Arizona	6	78	21	1
Utah		60	169	5
Nevada			60	9
			1	
Washington	2	6	651	52
Oregon		21	401	30
California	11	17	2,230	2
Total Median, 1943-48	144	16	3,068	52
250	8	2,770	23,784	43
Year to date, 13 weeks Median, 1943-48	2,242	100	55,771	1,110
Seasonal low week ends, Since seasonal low week Median, 1943-48	3,760	106	283,056	31,867
	(27th)		175,984	1,110
	July 10		190,206	2,548
	7,356		(35th)	(37th)
	11,326		Sept. 4	Sept. 18
			July 31	Sept. 4
			92,041	1,954
			335,449	4,052
			239,637	234,152
				239,637

* Period ended earlier than Saturday.

† New York City and Philadelphia only, respectively.

* Including cases reported as streptococcal infection and septic sore throat.

* Including paratyphoid fever; currently reported separately, as follows: Maine 1; New Jersey 2; North Carolina 1; South Carolina 1; Georgia 1; Texas 1; salmonella infections, not included, were reported as follows: New York 2.

Anthrax: Massachusetts 1; New York 2.

1 case.

Rocky Mountain spotted fever: Arkansas, delayed report, week ended Mar. 19, 1 case.

Smallpox: Deducted, Louisiana, week ended Feb. 19, 1 case; South Dakota, week ended Mar. 5, 1 case.

Alaska: No cases reported.

Territory of Hawaii: Influenza 36, measles 227, poliomyelitis 1.

FOREIGN REPORTS

CANADA

Provinces—Communicable diseases—Week ended March 12, 1949.—During the week ended March 12, 1949, cases of certain communicable diseases were reported by the Dominion Bureau of Statistics of Canada as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Chickenpox		17	8	284	624	34	49	45	312	1,373
Diphtheria				18						18
Dysentery, bacillary				4						4
Encephalitis, infectious					1					1
German measles				230	54		130	15	8	437
Influenza	16				31	14				61
Measles	137	33	245	315	195		162	275	209	1,571
Meningitis, meningococcal				1	2	1				5
Mumps	21	6	177	386	54	42		32	90	808
Scarlet fever	6		104	121	1	2		8	4	246
Tuberculosis (all forms)	8	16	107	24	13	8		16	55	247
Typhoid and paratyphoid fever			3	8		2				13
Undulant fever				1	1					2
Venereal diseases:										
Gonorrhea	4	10	101	51	25	16	31	55		293
Syphilis	5	2	55	36	11	11	9	10		139
Whooping cough	16	2	147	19	4	13	5			206

JAPAN

Notifiable diseases—4 weeks ended February 26, 1949, and accumulated totals for the year to date.—For the 4 weeks ended February 26, 1949, and for the year to date, certain notifiable diseases have been reported in Japan as follows:

Disease	4 weeks ended Feb. 26, 1949		Total reported for the year to date	
	Cases	Deaths	Cases	Deaths
Diphtheria	1,605	161	3,473	392
Dysentery, unspecified	138	36	301	98
Gonorrhea	14,229	36	29,279	
Influenza	216		391	
Malaria	85		199	5
Measles	9,195		15,406	
Meningitis, epidemic	107	28	216	53
Paratyphoid fever	116	4	332	8
Pneumonia	15,818		30,072	
Scarlet fever	316	7	837	17
Smallpox	3		4	
Syphilis	16,639		32,303	
Tuberculosis	32,215		62,512	
Typhoid fever	373	42	906	91
Typhus fever	25	1	58	1
Whooping cough	6,160		11,608	

NOTE.—The above figures have been adjusted to include delayed and corrected reports.

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**REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND
YELLOW FEVER RECEIVED DURING THE CURRENT WEEK**

NOTE.—Except in cases of unusual incidence, only those places are included which had not previously reported any of the above-mentioned diseases, except yellow fever, during recent months. All reports of yellow fever are published currently.

A table showing the accumulated figures for these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

Cholera

Burma—Bassein.—During the week ended March 12, 1949, 1 case of cholera was reported in Bassein, Burma.

India—Calcutta.—Cholera has been reported in Calcutta, India, as follows: Week ended March 12, 1949, 137 cases, 33 deaths; week ended March 19, 136 cases, 43 deaths; week ended March 26, 234 cases.

Indochina (French)—Cochinchina.—On March 20, 1949, 1 fatal case of cholera was reported in Cochinchina, French Indochina.

Plague

British East Africa—Tanganyika.—During the period February 1–20, 1949, 11 cases of plague, with 9 deaths, were reported in Tanganyika, British East Africa.

China—Wenchow.—During the period March 5–9, 1949, 3 cases of plague were reported in Wenchow, Chekiang Province, China.

India.—Plague has been reported in Calcutta and Cawnpore, India, as follows: In Calcutta, week ended March 19, 1949, 5 cases, week ended March 26, 18 cases; in Cawnpore, week ended March 12, 34 cases, 10 deaths, week ended March 19, 23 cases, 6 deaths, week ended March 26, 28 cases.

Rhodesia (Northern).—During the period February 1–17, 1949, 2 fatal cases of plague were reported in the Mankoya district of Barotseland Province, Northern Rhodesia.

Siam.—During the period February 20–March 12, 1949, 77 cases of plague, with 17 deaths were reported in Siam.

Smallpox

British East Africa—Nyasaland.—Smallpox has been reported in Nyasaland, British East Africa, as follows: In Lilongwe, week ended March 5, 1949, 19 cases, 4 deaths; in Blantyre, week ended March 12, 95 cases, 39 deaths (including cases not previously reported).

Burma.—For the week ended March 19, 1949, 12 cases of smallpox were reported in Rangoon, Burma, and 7 cases in Moulmein; for the week ended March 26, 17 cases were reported in Rangoon and 5 cases in Moulmein.

China.—Smallpox has been reported in China as follows: For the period March 1–10, 1949, Amoy 16 cases, Canton 28 cases; for the week ended March 26, Shanghai 19 cases.

Cuba—Habana.—Information received April 6, 1949, states that 1 additional case of smallpox has been reported in Habana, Cuba. This case is stated to have developed in the wife of the original patient. She had been in contact with him throughout the illness, and with all other contacts had been in isolation. The observation of these contacts ended April 6; and if no further cases developed before the end of the day, the quarantine of the contacts was to be ended as of that date.

India.—Smallpox has been reported in cities in India as follows: Week ended March 19, 1949, Ahmedabad 65 cases, Bombay 87 cases, Calcutta 62 cases, Madras 32 cases; week ended March 26, Bombay 118 cases, Calcutta 46 cases, Madras 28 cases.

Java—Batavia.—Information dated March 10, 1949, states that in the recent epidemic of smallpox in Batavia, 1,000 cases had been reported in Batavia and vicinity, up to March 3. The seriousness of the outbreak is stated to be considered increasing.

Transjordan.—During the week ended March 26, 1949, 15 cases of smallpox were reported in Transjordan, including 7 cases in Amman.

Typhus Fever

Chile—Santiago.—During the period March 4–20, 1949, 20 cases of typhus fever were reported in Santiago, Chile.

Ecuador.—During the period February 1–28, 1949, 28 cases of typhus fever (including murine type) were reported in Ecuador.

Italy—Naples.—During the week ended February 19, 1949, 5 cases of typhus fever with 2 deaths were reported in Naples, Italy.

Portugal—Lisbon.—During the week ended March 19, 1949, 1 case of typhus fever was reported in the city of Lisbon, Portugal.

Yellow Fever

Belgian Congo—Stanleyville Province.—On March 6, 1949, 1 death from yellow fever was reported in Titule Region, Stanleyville Province, Belgian Congo.

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